B_{12} Synporter
Another Brick in the Wall

iGEM Team Göttingen 2016
Department for Genomic and Applied Microbiology, University of Göttingen

About iGEM

The annual iGEM (**i**nternational **G**enetically **E**ngineered **M**achines) competition is the largest in the field of synthetic biology. It is operated by the iGEM Foundation that was established by the Massachusetts Institute of Technology. The participants are interdisciplinary student teams from all over the world who have to prove themselves in organizing and executing the entire project as well as finding financial support.

Background

Vitamin B_{12} (Fig. 1) is an **essential nutrient** for almost all organisms. However, its biosynthesis is confined to micro-organisms. Humans can mainly take it up via animal products or food supplements.

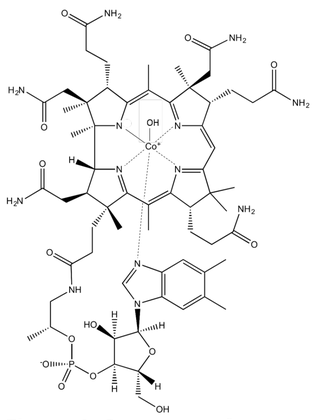

Figure 1: Structure of Vitamin B_{12} (Hydroxycobalamin)

Since the chemical production of vitamin B_{12} is very elaborate, production is facilitated by **genetically engineered microorganisms**. However, the produced Vitamin B_{12} is not exported out of the organism and must be harvested by cell lysis. This prevents a cost efficient production. To date, a natural cellular Vitamin B_{12} exporter is unknown.

Aim

We intend to design, construct and introduce a **synthetic Vitamin B_{12} exporter ("Synporter",** Fig. 2) into a production organism. Thereby, we aim for **higher yields in the industrial Vitamin B_{12} production** without a required cell lysis.

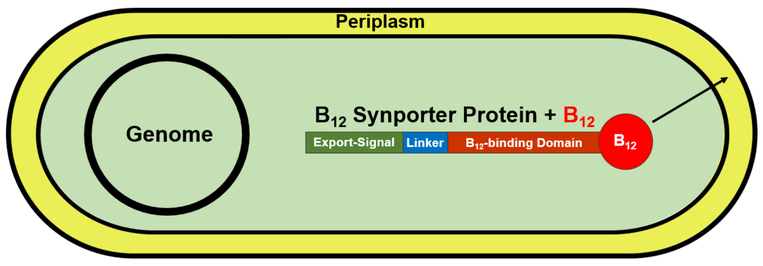

Figure 2: Model of the „Synporter" bound to Vitamin B_{12} inside a Gram-negative production organism.

Course of our Project

The B_{12} Synporter consists of a signal for a **twin arginine translocation (Tat)-mediated** export that is **linked to a B_{12}-binding protein.** Three different constructs are being tested (Tab. 1).

Table 1: Buildup of the B_{12} Synporter constructs.

Sequence	Original Peptide (*Species*): Function	Amino acids
Export signal	TorA (*E. coli*)	39
Linker	5 aminoacids post TorA export signal	5
B_{12} binding protein	α-subunit MutB *(P. shermanii)*: Methylmalonyl-CoA-Mutase; B_{12} co-factor	727
	BtuF *(E. coli* K12*)*: B_{12} import	265
	small subunit GlmS *(C. cochlearium)*: Glutamate mutase; B_{12} co-factor	136

What has been done already…

After checking their identity, the production strains *S. typhimurium TA100, S. blattae* and *R. planticola* were cultivated and their natural antibiotic resistances were determined. The vector pBAD202 was chosen for expression and the strains were made electrocompetent. The three different constructs for the B_{12} Synporter were designed and synthesized. (Fig. 3)

How we will proceed…

The constructs will be cloned into the vector (Fig. 4) and introduced into *E. coli* via electroporation to check for expression. This will be followed by the transformation of the production strains. The success of the Vitamin B_{12} export will be tested using a microbiological assay as well as spectrophotometry. Finally, we will present our results at the Giant Jamborée in Boston.

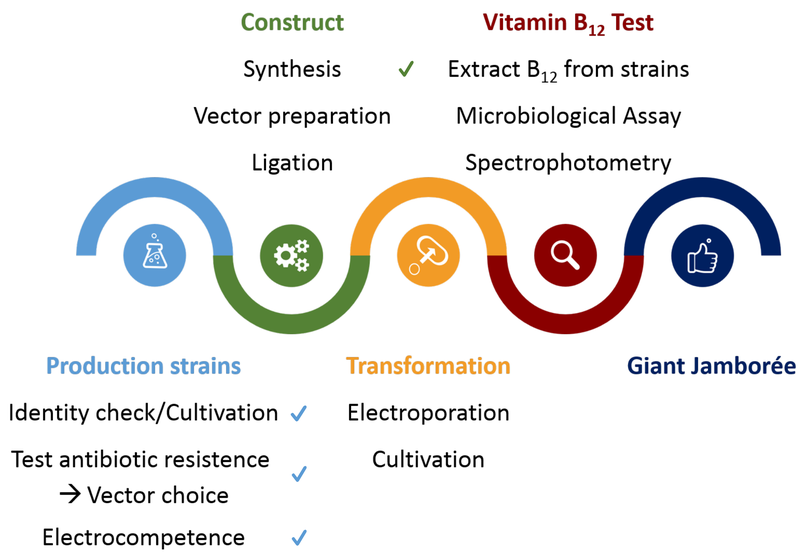

Figure 3: Course of our project. Check marks (✓) indicate completed tasks.

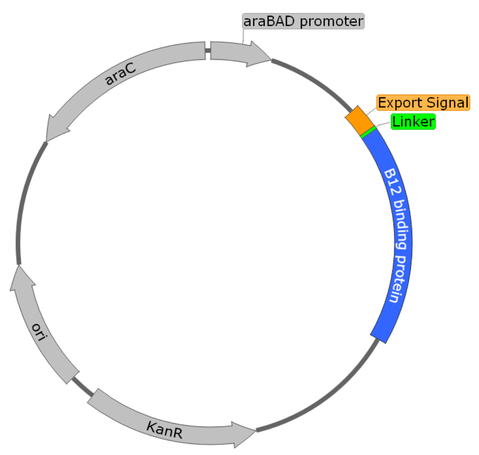

Figure 4: Model for a B_{12} Synporter construct inserted into the pBAD202 plasmid vector.
The vector includes the origin of replication (ori), a kanamycin resistance gene (*kanR*), the promoter of an L-arabinose operon (*araBAD* promotor) and the L-arabinose regulatory gene (*araC*).

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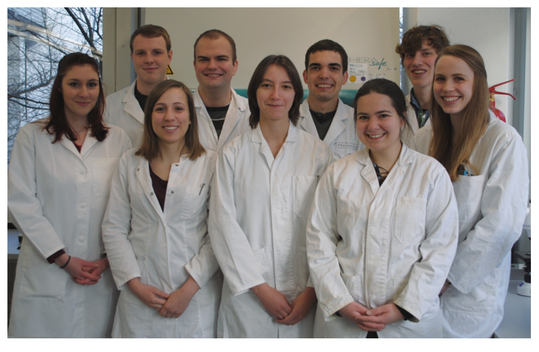

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